BACTERIAL TOXINS FOR RESEARCH AND INDUSTRY

PRODUCT INFORMATION

SNAPtide®, VAMPtide®, SYNTAXtide® and SNAP Etide®

FOR FLUOROMETRIC MEASUREMENT OF BOTULINUM NEUROTOXIN TYPES A, B, C and E

Seven immunologically distinct botulinum neurotoxins, designated types A, B, C, D, E, F and G are produced by the anaerobic bacterium *Clostridium botulinum*.[1] Synthesized as a single 150 kDa polypeptide chain in the bacteria, these neurotoxins are subsequently cleaved to produce two chains, a heavy chain and a light chain, which are linked by a disulfide bond.[2] For each toxin, the 50 kDa light chain is a zinc-dependent protease, which cleaves a single target protein essential for synaptic vesicle membrane fusion during neurotransmission. Botulinum neurotoxin types A and E specifically bind to and selectively cleave the synaptosome-associated protein, SNAP-25.[3] Botulinum neurotoxin types B, D, F and G specifically bind to and selectively cleave Synaptobrevin-2, also called VAMP-2.[4] Botulinum neurotoxin type C1 has been shown to cleave both SNAP-25 and the protein Syntaxin 1A. Cleavage of the target protein inhibits neurotransmitter release among neurons, which leads to muscular paralysis.[5] As little as 30-100 ng of the neurotoxin is potentially lethal to humans. Thus, detection of these toxins requires a highly sensitive and reliable assay. In addition, botulinum neurotoxins are increasingly useful clinically as the active ingredient in therapeutic agents. Sensitive and accurate potency assays are essential to assure toxin quantity, activity and safety in any given therapeutic formulation.

SNAPtide® (o-Abz/Dnp), product #520, SNAPtide® (FITC/DABCYL), product #521, and SNAPtide® flP6 (DABCYL/5-IAF), product #523, are synthetic peptides containing the native cleavage site for botulinum neurotoxin type A. VAMPtide® (o-Abz/Dnp), product #540, and VAMPtide® (FITC/DABCYL), product #541, are synthetic peptides containing the native cleavage site for botulinum neurotoxin type B. SYNTAXtide® (o-Abz/Dnp), product #560, is a synthetic peptide containing the native cleavage site for botulinum neurotoxin type C. SNAP Etide® (o-Abz/Dnp), product #550, is a synthetic peptide containing the native cleavage site for botulinum neurotoxin type E. All eight peptides are quenched fluorescent substrate peptides based on fluorescence resonance energy transfer (FRET). Initially the N-terminally attached fluorophore, o-aminobenzoyl (o-Abz), or fluorescein-thiocarbomoyl (FITC), is quenched by the C-terminally attached chromophore, 2,4-dinitrophenyl (Dnp), or DABCYL group. Product #523 contains the chromophore, DABCYL, on the N-terminal and the fluorophore, 5-IAF, on the C-terminal of the substrate. Cleavage of the substrate by the appropriate botulinum neurotoxin releases the fluorophore and full fluorescence is restored. The increase in fluorescence intensity is directly proportional to the amount of cleavage that has occurred and thus allows for accurate measurement of botulinum neurotoxin enzymatic activity.

Products #525 and #526 are control peptides for SNAPtide® (o-Abz/Dnp), product #520, and SNAPtide® (FITC/DABCYL), product #521, respectively. These peptides contain two amino acid substitutions and are not substrates for botulinum neurotoxin type A, however, they are ideal control peptides since they contain all of the sites for non-specific cleavage found in SNAPtide® #520 or SNAPtide® #521.

The FRET substrates can be used for development of highly sensitive and rapid *in vitro* methods with the potential for 1) detecting toxin contamination in food, clinical and environmental samples; 2) evaluating the potency of therapeutic agents containing botulinum neurotoxin types A, B, C and E; 3) detecting toxin neutralizing antibodies; as well as 4) screening and characterizing a large number of toxin inhibitors, which are potential therapeutic agents.

The hydrolysis of the o-Abz-peptidyl-Dnp substrates can be followed using an excitation wavelength of 320 nm and an emission wavelength of 420 nm. Hydrolysis of the SNAPtide® (FITC/DABCYL) and SNAPtide® flP6 (DABCYL/5-IAF) substrates are followed using an excitation wavelength of 490 nm and an emission wavelength of 523 nm.

Calibration peptides for SNAPtide® (product #520 and #521), which are the cleaved products containing only o-Abz (product #529) or FITC (product #528) at the N-terminal, and a calibration fluorophore for VAMPtide® (product #549), are also available.

All substrates are supplied as lyophilized powders. Lot analysis detailing purity and cleavage reaction conditions accompany each shipment.

These products are intended for research purposes only and are not intended for use in humans. For further information, please contact List Biological Laboratories, Inc.

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Ordering Information

Product No.	Description	Size
520	SNAPtide® Peptide Substrate (o-Abz/Dnp) for *C. botulinum* Type A Neurotoxin (U.S. Patent #6,504,006)	200 nmoles
521	SNAPtide® Peptide Substrate (FITC/DABCYL) for *C. botulinum* Type A Neurotoxin (U.S. Patent #6,504,006)	200 nmoles
523	SNAPtide® Peptide Substrate flP6 (DABCYL/5-IAF) for *C. botulinum* Type A Neurotoxin (U.S. Patent Pending #61/252,675)	200 nmoles
525	Control Peptide for SNAPtide® Product #520	200 nmoles
526	Control Peptide for SNAPtide® Product #521	200 nmoles
528	SNAPtide® Unquenched Calibration Peptide for Product #521	50 nmoles
529	SNAPtide® Unquenched Calibration Peptide for Product #520	50 nmoles
540	VAMPtide® Peptide Substrate (o-Abz/Dnp) for *C. botulinum* Type B Neurotoxin	200 nmoles
541	VAMPtide® Peptide Substrate (FITC/DABCYL) for *C. botulinum* Type B Neurotoxin	200 nmoles
549	VAMPtide® Calibration Fluorophore for Product #540	100 nmoles
550	SNAP Etide® Peptide Substrate (o-Abz/Dnp) for *C. botulinum* Type E Neurotoxin	100 nmoles
560	SYNTAXtide® Peptide Substrate (o-Abz/Dnp) for *C. botulinum* Type C Neurotoxin	200 nmoles

References

1. Simpson, L.L., Schmidt, J.J. and Middlebrook, J.L. (1988) *Methods Enzymol.*, **165,** 76-85.
2. Hatheway, C.L. (1989) Bacterial Sources of Clostridial Neurotoxins in *Botulinum Neurotoxin and Tetanus Toxin*, L.L. Simpson, ed., Academic Press, 4-24.
3. Schiavo, G., Rossetto, O., Catsicas, S., Polverino de Laureto, P., DasGupta, B.R., Benfenati, F., and Montecucco, D. (1993) *J. Biol. Chem.* **268,** 23784-23787.
4. Montecucco, C., Schiavo, G. (1995) *Q. Rev. Biophys.* **28,** 423-472.
5. Bigalke, H. and Shoer, L.F. (2000) In: *Handbook of Experimental Pharmacology*, Vol. 145 Bacterial Protein Toxins (K. Aktories and I. Just, Eds.) pp. 407-443, Springer-Verlag, Berlin.

POSTERS (available at www.listlabs.com)

1. Shine, N., Christian, T., Eaton, L., Crawford, K., (2005) *Rapid, Sensitive and Specific Assay to Measure the Endoprotease Activity of Botulinum Toxin Type A.*
2. Christian, T., Shine, N., Eaton, L., Crawford, K., (2005) *Comparison of Activity of Botulinum Neurotoxin Type A Holotoxin and Light Chain Using SNAPtide® FRET Substrates.*
3. Primak, Y., Christian, T., Shine, N., (2006) *SNAP Etide®, a FRET Substrate for Botulinum Toxin Type E.*
4. Christian, T., Shine, N., Crawford, K., (2007) *FRET Peptide Substrates for the Botulinum Toxins Type A, B and E and for Anthrax Lethal Factor.*
5. Christian, T., Endrukaite, E., Shine, N., (2009) *Ultra Sensitive HPLC Detection Assay for Botulinum Neurotoxin Type A. SYNTAXtide®, FRET Substrate for Botulinum Toxin Type C.*
6. Endrukaite, E., Christian, T., Shine, N., (2009) *FRET Peptide Substrate for Botulinum Neurotoxins Types A, B, C, and E.*
7. Shine, N., Suryadi, K., and Christian, T., (2010) *Evaluation of a Small Peptide Inhibitor and a Control Peptide for Botulinum Neurotoxin, Type A.*
8. Shine, N. and Suryadi, K. (2011) *New FRET Substrate for Botulinum Neurotoxin Type A.*